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CLAIMS

1. A method of imaging light from a specimen in which excitation light passes to the specimen via a confocal scanning system and light emitted by luminescence of the specimen passes in another direction via the scanning system to an image capture device having a sensor having discrete spatially distinct light sensitive regions, and the scanning system is operated so as to scan the whole of an area of interest of the specimen, wherein the scanning system, and the excitation light and/or the image capture device are controlled by a controller programmed to function as a state machine so that light emitted from the specimen is only incident on the image capture device sensor for a specific time period equal to that required for scanning the whole of the area of interest n times (where n is a whole number equal to or greater than 1).
2. A method as claimed in claim 1 wherein shutter means is provided which operates to prevent light reaching at least part of the image capture device sensor, except for the said specific periods of time during which the excitation light is incident on the specimen, for the purpose of reducing errors which can arise from light arising from phosphorescence, afterglow, stray reflections or other effects, from reaching the capture device sensor.
3. A method as claimed in claim 1 or claim 2 wherein the specimen is at least in part transparent and a plurality of images are formed by scanning the specimen in a plurality of different spaced apart planes.
4. A method as claimed in claim 3 wherein the different planes are produced by relative movement between the specimen and a scanning device forming part of the scanning system.

5. A method as claimed in claim 3 wherein the different planes are produced by movement of at least one part of an optical system forming part of the scanning system so that light is brought to a focus in the specimen at different spaced apart points, each point therefore defining the position of a focal plane of the scanning system.
6. A method as claimed in claim 4 or claim 5 wherein movement is restricted to periods during which excitation light is not incident on the specimen.
7. A method as claimed in claim 4 or claim 5 wherein movement is restricted to periods during which the image capture device is rendered insensitive to light.
8. A method as claimed in claim 4 or Claim 5 wherein the movement is continuous for the purpose of speeding up the scanning of a specimen.
9. A method as claimed in claim 8 wherein the continuous movement during the imaging results in blurring of the image, and the method includes the step of applying deconvolution to re-sharpen the image.
10. A method as claimed in any preceding claim wherein the excitation light is composed of light having two or more different wavelength(s).
11. A method as claimed in claim 10 wherein a single excitation light source is employed which comprises an acousto optic tuneable filter (AOTF) crystal and the wavelength of the emitted light is varied by altering the frequency controlling signal to the crystal as required.
12. A method as claimed in any preceding claim wherein the excitation light is pulsed.
13. A method as claimed in any preceding claim wherein the intensity of the incident excitation light is adjusted from one exposure to another by interposing neutral density filters, or opening or closing an iris diaphragm in the light path, adjusting the power to

the light source, or employing an attenuating element such as an AOTF or LCD shutter, or any combination thereof.

14. A method as claimed in any preceding claim wherein the specimen is illuminated by light at different wavelengths and the intensity is adjusted to produce a predetermined level of excitation intensity at the specimen for each wavelength.

15. A method as claimed in claim 14 wherein the adjustment produces a substantially similar level of intensity at the specimen for each different wavelength.

16. Apparatus by which light emitted from a specimen is imaged by an image capture device to produce a video signal for creating an image in a display device or for processing and analysis, comprising:-

- means for mounting the specimen,
- a light source for producing excitation light,
- a confocal scanning system adapted to direct excitation light in one direction towards, and thereby to scan an area of the specimen and also adapted to convey light emitted from the specimen as a consequence of the excitation light incident thereon, in another direction, which operates in use to scan typically repeatedly an area of interest of the specimen,
- an image capture device having discrete spatially distinct light sensitive regions on which light emitted from the specimen is focussed to form an image after being conveyed through the scanning system in the said other direction, and
- control means including a controller programmed to function as a state machine and adapted to control the scanning system, and excitation light and/or the image capture device so that light from the specimen is incident on the image capture device for a

specific time period equal to that required by the scanning system to scan the area of interest  $n$  times (where  $n$  is a whole number equal to or greater than 1).

17. Apparatus as claimed in claim 16 further comprising shutter means which in use is operated by signals from the control means to interrupt light from the excitation source except for when the specimen is to be illuminated wherein the shutter means comprises an acousto-optic element.
18. Apparatus as claimed in claim 17 further comprising second shutter means between the scanning system and the image capture device, which second shutter means is operated by signals from the control means so that in use light is prevented from reaching at least part of the image capture device sensor, except for the specific periods of time during which excitation light is incident on the specimen, for the purpose of reducing errors which could arise from phosphorescence, afterglow, stray reflections or light due to other effects, reaching the capture device.
19. Apparatus as claimed in any of claims 16 to 18 which further includes drive means adapted to move the specimen, the scanning system, or an element of an optical system within the scanning system, along a linear axis (the  $Z$  axis) so that in use the position of the plane can adjusted relative to the specimen.
20. Apparatus as claimed in claim 19 wherein in use the control system operates so as to restrict movement along the linear axis to periods during which light is prevented from reaching the image capture device.
21. Apparatus as claimed in claim 19 or claim 18 wherein the control system is operable to only produce movement along the linear axis during periods in which the excitation source light is inhibited or prevented from reaching the specimen.
22. Apparatus as claimed in claim 19 wherein the linear axis motion of the specimen, or scanning system, or element thereof, is continuous and wherein the apparatus further

comprises means by which deconvolution is applied to re-sharpen the image at the image capture device, or an image produced by signals from the image capture device, which is otherwise blurred due to the said continuous motion.

23. Apparatus as claimed in any of claims 16 to 22 wherein in use the wavelength of the excitation light is required to vary from one exposure to another, and the apparatus comprises two or more excitation light sources each producing excitation light of a different wavelength from the or each other source, and the control means is adapted in use to select the source to provide light of appropriate wavelength for each exposure.
24. Apparatus as claimed in any of claims 16 to 22 wherein in use the wavelength of the excitation light is required to vary from one exposure to another, the apparatus comprises a single source of excitation light which is adjustable to produce light of different wavelengths, and the control means is adapted to adjust the source to produce light having the required wavelength for each exposure.
25. Apparatus as claimed in any of claims 16 to 24 wherein the excitation light source is operable to produce light of more than one wavelength at the same time.
26. Apparatus as claimed in any of claims 16 to 25 wherein a single excitation light source is employed, the wavelength or wavelengths of the light emitted therefrom can be altered, and the control means is adapted to adjust the source to produce light of a desired wavelength or wavelengths wherein the light source is a laser light source which comprises an acousto-optical tuneable filter (AOTF) crystal, and the control means is adapted to provide signals to alter the frequency controlling signal to the crystal, to control the wavelength (or wavelengths) of the emitted light.
27. Apparatus as claimed in any of claims 16 to 26 wherein the excitation light source is operable so as to produce pulses of light.

28. Apparatus as claimed in any of claims 16 to 27 wherein the excitation light intensity is controlled by means of an attenuating element and the control means is adapted in use to control or position the attenuating element as appropriate.
29. Apparatus as claimed in claim 28 wherein the attenuating element is an AOTF or LCD shutter.
30. Apparatus as claimed in any of claims 16 to 29 wherein the control means is adapted to alter the intensity of the illumination so as to provide a predetermined intensity of illumination at the specimen for each wavelength, to remove variation in intensity from one wavelength to another as can occur due to inherent intensity variation as between one source and another or between different modes of operation of the excitation light source.
31. Apparatus as claimed in any of claims 16 to 30 wherein the control means is adapted to adjust the power to the excitation light source and/or control attenuation of light therefrom, from one exposure to another, to provide substantially constant intensity luminescence, to reduce variation in the intensity of the light incident on the image capture device sensor due to differing wavelengths of excitation light, or to render the light emitted due to luminescence of similar intensity irrespective of wavelength, or both.